THE PROTOZOA

Some Recent Protozoa Literature.—Waves of special enthusiasm sweep over the domain of biology as of other sciences; each leaves its mark, passes on and is followed by others. At one time it was the "section cutters" at another the "finger bowl brigade," at present it is genetics. Some investigators are independent enough to swim in quieter waters while some are so bold as to try to make an independent high-water mark long after the wave has passed. To the later group Hartmann and Prowazek must be assigned, for in a recent paper[1] they deal with the homologies of the centrosome, a problem which has never been solved and one that can not be regarded as obsolete, but which is no longer on the wave of biological enthusiasm.

Nor is the point of approach at all novel. They see in the nucleus and centrosome of the higher cell types only the reminiscence of a dual condition in protozoa. To be sure, they bring to bear a great fund of recently published observations, more particularly on protozoan cell structures, and they see in the metazoan nucleus and centrosome the outcome of dimorphic nuclei in these primitive animals. The two nuclei of the hypothetical ancestral form are not of the type suggested by Schaudinn in the early advocacy of this same theory (*Amœba binucleata*), but of the type seen in Trypanosoma, where trophonucleus and kinetonucleus are persistent morphological elements of the cell. They believe that the same dual arrangement is present in other protozoa; in some the kinetonucleus is reduced to a mere granule outside of the normal cell nucleus (as in the Centralkorn of the Heliozoa); in others the two nuclei are united to form an "amphinucleus," the one encased within the other as in the typical centronucleus. Here is the only really novel point in their discussion, and this can not be accepted, for to assign to the division center in a nucleus of the Euglena type the rôle of an independent nucleus is a *reductio ad absurdum*. The authors use a considerable area of printed matter to prove that these kinetoplasmic structures in protozoa are homologous with the centrosomes of higher forms, a point of view generally accepted more than a decade ago; and certainly nothing new is gained

[1] Hartmann and Prowazek. Blepharoplast, Karyosom und Centrosom. *Arch. f. Prot.*, X, 2–3.

by calling these division centers "nuclei." The one feature in support of this view is the presence of chromatoid material about the blepharoplast in Trypanosoma and in Paramœba, but even the truth of this is not generally accepted, Schaudinn's observations not having had sufficient confirmation to warrant universal acceptance. The authors' statement that chromatin material is likewise present in the great sphere of Noctiluca is not true. On the whole this conception of blepharoplast, karyosome and centrosome gives an inadequate summary of the striking recent advances in protozoan morphology and leaves the problem of the origin of the metazoan centrosome very much as it was ten years ago.

The pioneer work of Schaudinn's upon which this conception of Hartmann and Prowazek's is based has not been fully and satisfactorily confirmed, while much of it has been denied. Novy, for example, has continually fought against the double intra- and extra-cellular life of *Trypanosoma noctuæ* and now Moore and Breinl,[2] working with different kinds of *Trypanosoma,* find that the nuclei of *T. gambiense*, *T. brucei* and *T. equinum* all conform to the centronucleus type and that the blepharoplast or kinetonucleus (Woodcock) arises in the latent bodies by halving of the intra-nuclear division center and without any accompanying chromatin such as Schaudinn described in the case of *T. noctuæ*. Passing over the fact that the present authors somewhat stultify themselves on the perfection of their technique and give an ungenerous blanket criticism of all others who have worked upon the morphology of trypanosomes, it must be admitted that their criticism is to a certain extent justified, for the majority of observers have made too free use of the dry smear method. The so-called chromosomes of the trypanosomes, for example (not chromosomes at all in the strict sense), are interpreted by Moore and Breinl as irregular masses of chromatin which may assume any form under the rough processes of the dry method of fixation. This may or may not be true, but at any rate the figures given by the English authors and representing the finer structures of these nuclei do not inspire confidence in the methods which they themselves advocate, although they are, indeed, well tried and recognized methods.

[2] Moore and Breinl. Cytology of the Trypanosomes. *Annals of the Liverpool School of Tropical Medicine,* 1, No. 3.

Apart from this question of technique, upon which the last word is not yet given, Moore and Breinl bring forward evidence which throws a new light on the life history of *T. gambiense,* the cause of sleeping sickness. They find a phase in the life history where the nucleus, surrounded by a small bit of protoplasm, is left over after the bulk of the trypanosome has degenerated. This nucleated bit, which they name the "latent body," is stored up in the spleen and bone marrow of the experimental animal (rat), ultimately reappearing in the circulation where a new, young trypanosome arises from it. They find no evidence of trimorphic differentiation which Schaudinn first described for *T. noctuæ,* but they call attention to the fact that a complete series of sizes of trypanosoma may be selected, and claim that the indifferent, male, and female, forms are only arbitrarily chosen individuals from such a series.

Another interpretation is given to the trypanosomes with long chromatin bars such as Prowazek in the case of *T. lewisi* regarded as male forms. The English observers introduce a new hypothesis to account for this, viz., that it represents a type of autogamous conjugation. The bar is of kinoplasmic material growing out from the blepharoplast to the nucleus, where a portion of its substance, as they believe, unites with the nucleus. The suggestion is ingenious and, in view of the constantly growing evidence in favor of autogamy in other kinds of protozoa (Entamœba, Actinosphærium, *Amœba proteus,* etc.), must be taken into account.

On *a priori* grounds it would certainly seem that if conjugation among trypanosomes is a normal part of the life history, and there is no reason to believe it absent, it would be more frequently observed and there would be no uncertainty about it. Its infrequency and the doubt existing in regard to the observations that have already been made lead us to suspect that conjugation of some obscure type occurs here. In flagellated protozoa where conjugation of the ordinary type is characteristic, the periodicity of conjugation is one of the most noteworthy features. This is well illustrated in a timely article by C. Clifford Dobell on the life history of a simple Peranema-like flagellate which he names *Copromonas subtilis.*[3] The flagellate is a com-

[3] Dobell, C. Clifford. The Structure and Life History of *Copromonas subtilis* nov. gen. et nov. sp., a Contribution to our Knowledge of the Flagellata. *Q. J. M. S.*, No. 205, 1908.

mon parasite of the rectum of frogs and toads and grows readily, the author observes, in rectal contents with normal salt. Conjugation between similar organisms (isogametes) occurs from the seventh to the ninth day of such a culture, the result being either an encysted form (permanent cyst) or a motile form which reproduces by division for several generations and then encysts.

The trypanosomes evidently present no such simple life history as this which Dobell describes and, although accumulating evidence makes it probable that all stages are confined to the single host in the greater number of cases at least, every description of conjugation thus far published is so fantastic as to arouse suspicion. What is true of trypanosomes is even more characteristic of spirochætes, a field of research so difficult that very few have had the hardihood to publish accounts of conjugation, and needless to say none that has been published is acceptable. The latest on Spirochæta is a paper by H. B. Fantham on the relatively large spirochæte of the oyster and clam.[4] This *Spirochæta balbianii*, which was monographed by Perrin a couple of years ago, is interesting in having a central helix of chromatin which is spirally wound, and upon which larger granules of chromatin are suspended at intervals. This represents an intermediate condition, so far as the nucleus is concerned, between the isolated granules of chromatin in bacteria and in certain forms of Spirochæta (*e. g., S. obermeieri*), and the formed nuclei of higher types of protozoa. Reproduction both of this form and of *S. anodontæ* is by longitudinal and occasionally transverse division, both types occurring according to the author. Conjugation in no form was observed. Like the majority of recent writers on the spirochætes, Fantham proposes a new group for them intermediate between the protozoa and the bacteria. He suggests that they be made a new "class" of organisms under the name "Spirochætacea." Such innovations, however, can do no good and it is far better not to further confuse an already mixed up classification. When the full life history of the genus (or genera) of Spirochæta is known it will be time enough to change the classification.

These flagellates are not the only forms of protozoa over

[4] Fantham, H. B. *Spirochæta* (*Trypanosoma*) *balbianii* (Certes) and *Spirochæta anodontæ* (Keysselitz). Their Movements, Structure and Affinities. *Q. J. M. S.*, No. 205, 1908.

which there is much discussion and controversy at the present time. The rhizopods offer quite as extensive a field for divergent opinions and here again it is mainly in connection with the parasitic types. The life histories of the ordinary forms are being slowly established and with this basis the parasitic forms should be comparatively simple to work out. The one remaining important step to be made in working out the life history of the common rhizopod *Arcella vulgaris* has quite recently been taken by W. Elpetiewsky,[5] and it is fitting that it should have been made in Hertwig's laboratory, where the first important steps were taken. The author finds that gametic nuclei are formed from the distributed chromidia in the way described by Schaudinn for Centropyxis, and by Schaudinn and Lister for Polystomella. Macrogametes and microgametes are formed and conjugation of two such anisogamous swarmers was followed step by step. He found furthermore, that Arcella reproduces also by the formation of pseudopodiospores, and that these develop fine heliozoa-like radiating pseudopodia, upon the ends of which they roll about for a period of from two to three hours. This observation is interesting in the light of the pos sible origin of the lobose rhizopods from the heliozoa.

It is not quite so simple a matter to accept the latest work on the parasitic rhizopods and we entirely disagree with Prowazek in his conception of the so-called group Chlamydozoa. In this proposed new group of protozoa which he would place intermediate between the protozoa and the bacteria, Prowazek places the majority of the recently contested forms of disease-produ cing organisms. The disputed organisms of variola, vaccinia, scarlet fever, trachoma, rabies, *Molluscum contagiosum,* and some others of less importance are all grouped together here apparently without regard to their morphology or effects upon the host. All of these organisms are regarded as extremely small cell parasites which are made conspicuous by reason of a more or less thick secretion of nuclear material about them, the name of the proposed group being based upon this characteristic (χλαμυς—mantle).

In this supposition the author takes a great deal for granted and begs two very important questions, first, that the inclusions

[5] W. Elpetiewsky. Zur Fortpflanzung von *Arcella vulgaris* Ehr. in *Arch. f. Prot.,* X, No. 2–3, 1907.

[6] S. Prowazek. Chlamydozoa. In *Arch. f. Prot.,* X, No. 2–3, 1907.

are organisms, and second, if organisms, that the bulk of their substance is to be traced to nuclear secretions. The limits of the present reference will not permit an extensive analysis of Prowazek's different assumptions, but one or two matters may be pointed out as showing his method of treatment, and incidentally his ignorance or, possibly, disregard, of careful work of others. The organism of rabies, for example, exists, as do all rhizopod protozoa in many different sizes, and one form of the organism is extremely minute. This small phase is characteristic of the so-called "fixed virus" and has been entirely overlooked by Prowazek despite the accurate and detailed work of Dr. A. W. Williams and others. In another phase the organism is quite large and characteristically amœboid in form. Prowazek finds none of these larger forms in centrifuged virus and concludes that the intra-cellular phase is absent in the virus thus treated, and since he failed to see these forms in the fixed virus he further concludes that the bulk of the Negri body in rabies must be a nuclear secretion and that the organisms are the minute brightly staining points (chromatin) of the larger forms which are actually present in the fixed virus and in centrifuged virus, but invisible. In this conclusion he shows not only a total disregard for what other competent workers have done, but a surprising ignorance of the actual structure of the Negri body.

Apart from special criticisms which might be carried out for each of the diseases mentioned, the general criticism may be made that it is not good zoology to create a group in classification while there is still some doubt as to the organisms being living things; and it is not good cytology to assume an entirely new function (of specific secretions) in various cells in response to such questionable organisms. The present critic believes, indeed, that these questionable structures are organisms, and organisms belonging to the rhizopod group of protozoa, but not to any group with the characteristics of the proposed "Chlamydozoa."

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EXPERIMENTAL ZOOLOGY

The Determination of Sex in Frogs.—Few results in experimental biology have been more puzzling than those involving the question of the determination of the sex of the frog. The earliest